REPORT

Homozygous Mutation in *SPATA16* Is Associated with Male Infertility in Human Globozoospermia

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Globozoospermia is a rare (incidence <0.1% in male infertile patients) form of teratozoospermia, mainly characterized by round-headed spermatozoa that lack an acrosome. It originates from a disturbed spermiogenesis, which is expected to be induced by a genetic factor. Several family cases and recessive mouse models with the same phenotype support this expectation. In this study, we present a consanguineous family with three affected brothers, in whom we have identified a homozygous mutation in the spermatogenesis-specific gene *SPATA16*. This is the first example of a nonsyndromic male infertility condition in humans caused by an autosomal gene defect, and it could also mean that the identification of other partners like *SPATA16* could elucidate acrosome formation.

Approximately 15% of couples are confronted with the inability to conceive after 2 years of unprotected intercourse.[1] In about half of these cases, infertility is due to the inability of the male partner to produce spermatozoa of sufficient number (oligozoospermia), adequate motility (asthenozoospermia), or normal morphology (teratozoospermia) or to combinations of these defects. Globozoospermia (MIM 102530) is a rare but severe teratozoospermia, characterized by ejaculates consisting completely of round-headed spermatozoa that lack an acrosome or, in partial globozoospermia, containing a variable proportion (20%-90%) of acrosomeless spermatozoa.[2–4] Men that are affected with total globozoospermia are infertile, and even the application of intracytoplasmic sperm injection (ICSI) has met with disappointingly low success rates.[2] Globozoospermia originates from a disturbed spermiogenesis, and, although the underlying cause is still unknown, a genetic contribution appears to be supported by several familial case reports[5–7] and by three recessive mouse models involving *CSNK2A2* (MIM 115442), *HRB* (MIM 600862), and *GOPC* (MIM 606845).[8–10] However, no causative gene mutations have been identified in these orthologues or any other human genes to date.[11,12] We describe a family with three affected brothers, in whom we have identified a homozygous mutation in the spermatogenesis-specific gene *SPATA16* (MIM 609856). To our knowledge, this is the first example of a nonsyndromic male infertility condition in humans caused by a single gene defect.

We investigated an Ashkenazi Jewish family with six brothers (three affected and three healthy) and four sisters (fig. 1*D*) that was identified at the Centre for Reproductive Medicine of the Dutch-Speaking Brussels Free University. The three unaffected brothers fathered seven, six, and five children, respectively, but the three affected brothers were childless and presented with a fertility disorder due to oligoasthenoteratozoospermia, showing the characteristics of total globozoospermia, such as roundheadedness and acrosomelessness, as shown by acrosin (MIM 102480) staining in figure 1*A*. No known consanguinity was reported, although the family belonged to an isolated Jewish population. A normal karyotype and no Y-chromosome microdeletion were found. In two brothers, ICSI was performed, but fertilization was poor, and no pregnancy occurred.

We performed a genomewide scan analysis of all six brothers, using a 10K SNP array (Affymetrix GeneChip). Regions of homozygosity were defined by the presence of >25 consecutive homozygous SNPs. Large regions of homozygosity were observed in all six individuals (tables 1 and 2), indicating consanguinity in the second or third degree in the family. Therefore, we considered this family to be consanguineous and expected the pathology to be autosomal recessive. We identified a unique region of haplotypic identical homozygosity shared by all affected brothers, in which the healthy brothers were heterozygous. The smallest region of overlap spanned 17 Mb of chromosome 3q26 (167054711–184087390). This region

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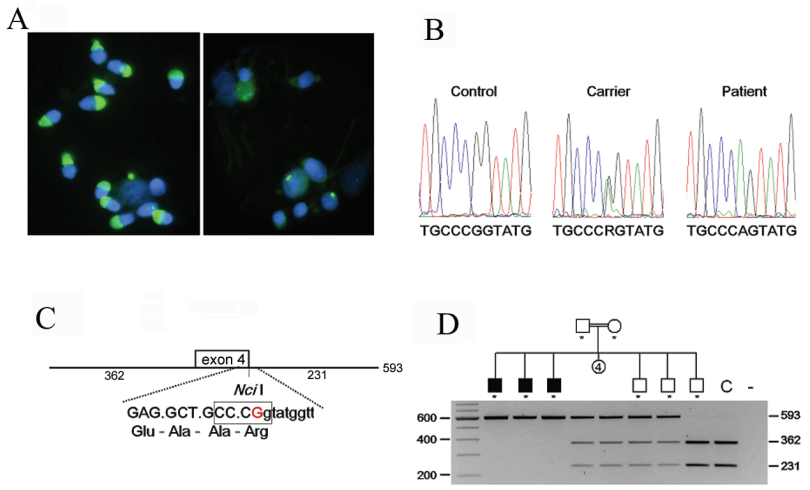

Figure 1. Family with globozoospermia and a mutation in the *SPATA16* gene. *A,* Sperm morphology. Fluorescent acrosin (*green*) staining (with fluorescein) of acrosomes and 4′,6-diamidino-2-phenylindole (*blue*) staining of nuclei. On the left is a sample from a fertile control, in which the most important content of the acrosome (acrosin) is clearly and abundantly present; on the right is a sample from a patient. Sperm morphology and acrosome structures are severely disrupted in patient cells. Remnants of acrosin staining were observed for some deformed sperm cells, but most signals represent nonspecific acrosin staining in the leukocytes. *B,* Chromatograms of the mutation. Shown are the sequences from a control sample, the heterozygous father, and one of the patients. *C,* The *Nci*I recognition site (5′-CCCGG-3′) is lost because of the G→A mutation at the last nucleotide of exon 4. (The *Hpa*II recognition site is not shown but overlaps at 5′-CCGG-3′). This mutation predicts a R283Q amino acid substitution, as well as the disruption of the 5′ splice site of intron 4. *D,* Pedigree of the Ashkenazi Jewish family in this study. The order of the 10 siblings is arbitrary. The segregation of the mutation was studied by *Nci*I digestion of a PCR amplification of exon 4 and its flanking sequences. The first lane is the marker lane. As asterisk (*) indicates tested individuals. The two parents and two siblings are heterozygous for the mutation. The three affected males are homozygous, and one unaffected male and a control (C) are not carriers of the mutation.

contains ~50 known genes in the UCSC Genome Browser. We selected the *SPATA16* gene (spermatogenesis-associated 16, also known as "*NYD-SP12*") as the most plausible candidate gene, because recent studies showed that *SPATA16* is specifically expressed in human testis and that the mouse ortholog is primarily expressed in the spermatocyte and spermatids.[13] Localization in the Golgi apparatus and the shift with Golgi vesicles to the acrosome was observed in round and elongated spermatids by use of a SPATA19-GTP (green fluorescent protein) fusion protein, strongly suggesting a role for the SPATA16 protein in acrosome formation during spermiogenesis.[14] *SPATA16* is composed of 11 exons encoding a highly conserved protein of 65 kDa (569 aa), which contains a tetratricopeptide repeat (TPR [MIM 602259]) domain. Sequence alignment (by use of ClustalW 1.81 [SDSC Biology Workbench]) (fig. 2) shows that *SPATA16* is highly conserved across mammals, exhibiting an identity rate varying from 77% (mouse) to 96% (chimpanzee) (NCBI Blast) (table 3). The conservation is even higher (92% and 98% in mouse and chimpanzee, respectively) for the TPR domain, a protein-protein interaction domain commonly but exclusively found in cochaperone proteins.[15]

Sequence analysis of one of the affected sons revealed a homozygous sequence variation in exon 4 (c.848G→A), which disrupts a *Nci*I or an *Hpa*II recognition site (fig. 1*C*). Restriction-enzyme analysis revealed that the three affected brothers are homozygous and that the two parents and two healthy brothers are heterozygous for the mutation. The third unaffected brother appeared to be homozygous for the wild-type sequence (fig. 1*D*). The c.848G→A nucleotide variation is not known in any SNP database and was not identified in 231 controls, including 151 random controls of both sexes and 80 fertile males.

The mutation predicts an amino acid change of a highly conserved residue (p.R283Q) located at the C-terminal end of the highly conserved TPR domain. In addition, the

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics.*

Figure 2. Sequence alignment of *SPATA16*

Table 1. Results of the SNP Array for the Three Affected Brothers

Chromosome 3 Position	SNP	Genotype Call in Sibling 1	2	3
165727121	SNP_A-1508753	AA	AA	AA
167054711	SNP_A-1512676	AB	AB	AB
167631594	SNP_A-1508627	AA	AA	AA
167801377	SNP_A-1519252	AA	AA	AA
169050879	SNP_A-1511126	BB	BB	BB
169140975	SNP_A-1509483	AA	AA	AA
169748882	SNP_A-1518417	BB	BB	BB
170222552	SNP_A-1518965	BB	BB	BB
170229669	SNP_A-1509719	BB	BB	BB
170266790	SNP_A-1519387	BB	BB	BB
170341708	SNP_A-1513150	BB	BB	BB
170440150	SNP_A-1513458	AA	AA	AA
170815817	SNP_A-1516975	AA	AA	AA
171062673	SNP_A-1514614	No Call	AA	No Call
171141972	SNP_A-1508020	BB	BB	BB
172368188	SNP_A-1510308	AA	AA	AA
172643136	SNP_A-1517656	BB	BB	BB
172771949	SNP_A-1509479	AA	AA	AA
172933454	SNP_A-1509435	AA	AA	AA
172933529	SNP_A-1509382	BB	BB	BB
173575998	SNP_A-1516388	BB	BB	BB
173580444	SNP_A-1514288	BB	BB	BB
173580729	SNP_A-1514241	AA	AA	AA
174671975	SNP_A-1507368	AA	AA	AA
175782878	SNP_A-1508795	BB	BB	BB
176537318	SNP_A-1511779	AA	AA	AA
176610712	SNP_A-1509801	BB	BB	BB
176827123	SNP_A-1511813	AA	AA	AA
177333023	SNP_A-1517824	AA	AA	AA
177621541	SNP_A-1518682	BB	BB	BB
177708137	SNP_A-1510834	AA	AA	AA
177723240	SNP_A-1516780	BB	BB	BB
178105620	SNP_A-1516425	AA	AA	AA
178105781	SNP_A-1515959	No Call	BB	No Call
179324469	SNP_A-1517000	No Call	AA	No Call
179597123	SNP_A-1516215	AA	AA	AA
179597352	SNP_A-1516746	AA	AA	AA
179636158	SNP_A-1512810	BB	BB	BB
179706441	SNP_A-1510950	BB	BB	BB
179839122	SNP_A-1514173	BB	BB	BB
180413909	SNP_A-1511671	AA	AA	AA
180729255	SNP_A-1508156	BB	BB	BB
181028753	SNP_A-1512457	BB	BB	BB
181180943	SNP_A-1507868	BB	BB	BB
181251555	SNP_A-1512336	AA	AA	AA
181298402	SNP_A-1513747	AA	AA	AA
181506449	SNP_A-1517808	BB	BB	BB
181552274	SNP_A-1509494	BB	BB	BB

NOTE.—Several large areas of shared haplotype were identified, indicating consanguinity. The area of shared haplotype on which we concentrated is shown.

c.848G→A mutation affects the last nucleotide of exon 4 (fig. 1*C*) and, therefore, may disrupt the 5′ splice site of intron 4. Three different splice-site prediction models predicted that the mutation disrupts this splice site (table 4). Unfortunately, the SPATA16 protein presents a testis-restricted expression, and we were not allowed to use fresh sperm cells or to perform a biopsy in these religious patients to verify the predicted aberrant splicing in vivo. Therefore, minigene constructs were made that consisted of two constitutive β-globin exons surrounding a 420-bp fragment containing either the wild-type or the mutated form of exon 4 and the flanking intronic sequences of *SPATA16*. These minigene constructs were transfected into COS1 or HeLa cells, and transcripts were analyzed by RT-PCR 24 h after the transfection (fig. 3*A*). As shown in figure 3*B*, wild-type exon 4 is invariably included in the final mRNA, as confirmed by the sequencing of the PCR product. In sharp contrast, the mutated exon gives rise to two aberrant splicing forms, as shown by cloning and sequencing of these PCR products. The most prominent, larger product is the result of the use of a splice site situated in the β-globin intron used for the minigene construct. The weaker, smaller product corresponds to the use of a cryptic splice site situated 18 bp upstream of the normal splice site. These aspecific products are likely the result of the very short intron sequences in the minigene construct. Such products are often seen in exon-trapping experiments in the absence of a bona fide splice site and are indicative of the occurrence of exon skipping due to the mutation.[16,17] Importantly, we did not detect any transcript containing the correct junctions from the mutated exon 4, indicating that the mutation hinders normal splicing.

The first and critical step of exon inclusion is the binding of the U1 small nuclear ribonucleoprotein (SnRNP) splicing factor to the 5′ splice sites.[18] To confirm that the mutated *SPATA16* exon 4 is not recognized by the splicing machinery, we checked its binding to the U1 SnRNP by psoralen-mediated UV crosslinking. Whereas binding of U1 SnRNP to wild-type DNA was readily detected, this was not observed when DNA carrying the c.848G→A mutation was used as template (fig. 3*C*). The identity of the U1 SnRNP was confirmed by RNAse H treatment by use of an oligodeoxynucleotide complementary to positions 1–15 of U1 SnRNA.[19] Therefore, the results of the bioinformatic prediction, the minigene, and the U1 binding strongly suggest that the c.848G→A mutation leads to inappropriate splicing of exon 4 and, therefore, the disruption of the TPR domain.

SPATA16 was also analyzed in 29 patients with globozoospermia, including 6 familial cases involving 14 patients. Of the 29 patients, 12 presented with total globozoospermia, and 17 with partial globozoospermia. None of them presented with any variation in the *SPATA16* sequence, with the exception of three known polymorphisms and two point mutations that did not segregate with the disease (table 5).

Table 2. Areas of Shared Haplotype and Shared Homozygosity

Shared Haplotype				Shared Homozygosity			
Chromosome and Start Position	Stop Position	Fragment Length (bp)	No. of SNPs[a]	Start Position	Stop Position	Fragment Length (bp)	No. of SNPs[a]
1:							
33955677	36556426	2,600,749	15				
117399167	119296010	1,896,843	11	117866019	119296010	1,429,991	6
151489481	156376556	4,887,075	11	151489481	156376556	4,887,075	11
160643582	162290883	1,647,301	14				
194965564	198362174	3,396,610	10				
2:							
19716714	34386124	14,669,410	55				
54483803	57217291	2,733,488	14				
59155900	65801963	6,646,063	11				
65801963	107509848	41,707,885	91				
113469814	115959977	2,490,163	12				
212719583	215032622	2,313,039	10				
224103332	226044921	1,941,589	16				
234216839	239151790	4,934,951	14	234719101	239151790	4,432,689	8
3:							
653347	3558063	2,904,716	12				
100634082	103786422	3,152,340	10				
113839242	127152417	13,313,175	48				
165655532	184087390	18,431,858	49	167054711	184087390	17,032,679	47
184087390	190712906	6,625,516	26				
4:							
173190930	176925273	3,734,343	10	173530324	176925273	3,394,949	8
181334851	191091333	9,756,482	48				
5:							
120723042	122283900	1,560,858	11	121110284	122283900	1,173,616	7
134671015	139500740	4,829,725	15				
6:							
28766533	31094058	2,327,525	10	29479394	31094058	1,614,664	6
46333713	47986997	1,653,284	14	46824038	47986997	1,162,959	5
96879221	102207593	5,328,372	12				
105174572	108446020	3,271,448	10				
108446020	52383480	43,937,460	169				
7:							
77627148	131407468	53,780,320	169				
8:							
53838124	57959516	4,121,392	15				
72728798	76200122	3,471,324	10				
9:							
507715	13460671	12,952,956	85	12445236	13460671	1,015,435	6
72760108	75532057	2,771,949	10	73265909	75532057	2,266,148	7
75532057	90138831	14,606,774	56				
10:							
63884288	66164664	2,280,376	10				
66164664	68113302	1,948,638	10				
91501439	105835217	14,333,778	47				
14:							
19490525	22620727	3,130,202	20				
31508337	33265230	1,756,893	10				
36021565	37647284	1,625,719	10				
15:							
21490270	23325412	1,835,142	13				
23325412	59077153	35,751,741	127				
79723090	84387340	4,664,250	12				
16:							
22705353	50026393	27,321,040	24				
17:							
28942222	34693022	5,750,800	10	29183029	34693022	5,509,993	9
66657008	72151125	5,494,117	10				
18:							
63746898	66906214	3,159,316	13	64766169	66906214	2,140,045	9
20:							
38790879	43185196	4,394,317	10				
21:							
18764912	21113081	2,348,169	11	18764912	21113081	2,348,169	11
29925652	31842275	1,916,623	12				
36234195	37974748	1,740,553	10	36447405	37974748	1,527,343	7

NOTE.—In the left part of the table, a selection of the areas of shared haplotype (those with (>9 SNPs) is displayed. In the right part of the table, the regions of shared homozygosity (with >4 SNPs) that lie within are shown.

[a] Number of SNPs that form the area of shared haplotype or homozygosity.

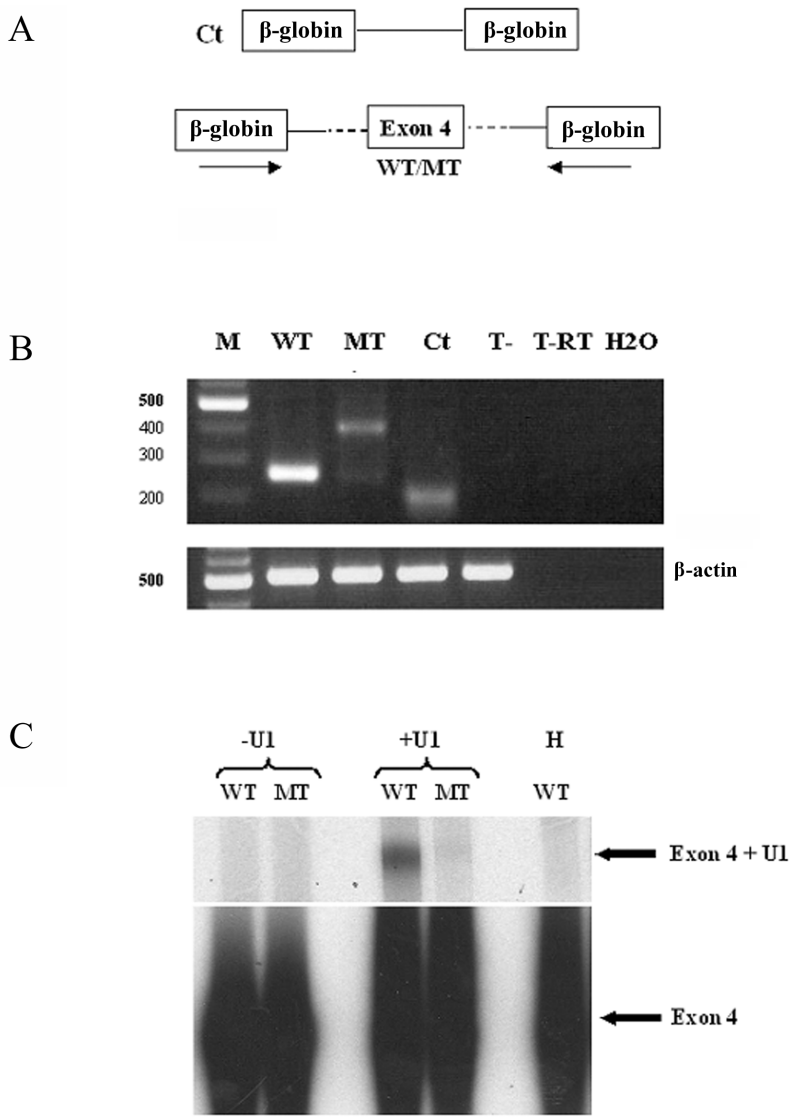

Figure 3. Mutated donor splice site of *SPATA16* intron 4 and U1 SnRNP binding to wild-type (WT) and mutant (MT) donor splice sites. *A,* Overview of the used prediction sites. *B,* Minigene constructs used to test the splicing of exon 4. T- = construct without exon; T-RT = construct without exon in RT-PCR. *C,* Gel showing that wild-type exon 4 is invariably included in the final mRNA. In sharp contrast, the mutant exon gives rise to two aberrant splicing forms. M = marker lane. *D,* U1 SnRNP binding analyzed by psoralen-mediated UV-crosslinking experiments, revealing that mutant exon 4 is not recognized by the splicing machinery, whereas wild-type exon 4 is clearly recognized. The identity of the U1 SnRNP was confirmed by RNAse H treatment with use of an oligodeoxynucleotide complementary to nucleotide positions 1–15 of U1 SnRNA.

This is, to our knowledge, the first description of a gene involved in the pathogenesis of human globozoospermia. The data strongly suggest that the identified homozygous mutation in *SPATA16* causes globozoospermia in three of six brothers in the family studied, which allows us to state that globozoospermia can be a genetic trait with an autosomal recessive mode of transmission. The *SPATA16* protein localizes to the Golgi apparatus and to the proacrosomic vesicles that are transported to the acrosome in round and elongated spermatids during spermiogenesis. Our observations support the hypothesis of a crucial role for *SPATA16* in acrosome formation.[14] The strongest protein conservation is seen in the TPR domain, which is disrupted in these cases of globozoospermia. The TPR domain is known to mediate protein-protein interactions and assembly of multiprotein complexes. Study of the x-ray structure revealed that the TPR domain adopts a helix-turn-helix arrangement, with the ability to associate with

Table 3. Identity Rates among Species for the *SPATA16* Sequence and the TPR Domain of the Protein

Species	Identity with Human (%)		Positives[a] (%)		Gaps[b] (%)	
	SPATA16	TPR Domain	*SPATA16*	TPR Domain	*SPATA16*	TPR Domain
Homo sapiens	100	100	100	100	0	0
Bos taurus	87	94	92	97	0	0
Canis familiaris	83	97	89	97	0	0
Macaca fascicularis	95	97	97	97	0	0
Mus musculus isoform 1	77	92	86	96	0	0
M. musculus isoform 2	78	92	87	96	0	0
Pan troglodytes	96	98	98	98	0	0
Rattus norvegicus	80	93	87	96	0	0

[a] Amino acid positive-match score.

[b] Space introduced into an alignment to compensate for insertions and deletions in one sequence relative to another. In our search, the gaps did not exceed 0.5%.

other α-helical structures. A possible interacting protein may be from the gene *GOPC,* a Golgi-associated protein containing coiled-coil motif α-helices,[10] or from the HIV-1 rev binding protein gene (*HRB*), which also localizes to the Golgi complex.[9] Both these genes are involved in the pathogenesis of globozoospermia in mouse models. Finally, it is worth noting that *SPATA16* contains six casein kinase II phosphorylation sites and that the casein kinase IIa′ is the most abundant casein kinase in the testis,[8] for which the knockout model shows acrosome and other morphological defects.[20] Moreover, the existence of several candidate genes[8–10] suggests genetic heterogeneity in human globozoospermia, which could be a reason why we did not find other patients with a gene alteration in *SPATA16*. Noteworthy as well is the fact that the heterozygous mouse models show no sperm abnormalities. This indicates that mutation carriers should have normal fertility. In this family, this seems to be the case, since the father and two heterozygous brothers have fathered 10, 7, and 6 children, respectively. In two of the affected brothers, ICSI was performed to induce fertilization and pregnancy, but without success. This is in accordance with the literature, which shows that ICSI enables oocyte fertilization, but with low fertilization rates in about half of the cases.

Since male infertility does not respect the canonical rule of genetics, the determination of inheritance patterns and the elucidation of genetic causes are complicated. Several genetic factors have been described that affect male fertility,[21] but these give rise to more complex phenotypes. However, the patients in this study did not show any mental or physical abnormalities—in particular, no andrological abnormalities—in addition to their aberrant semen analysis. Thus, the mutation in *SPATA16* that we found in this study appears to present a human gene in which mutations give rise to male infertility without any associated other anomalies.

Further studies of other patients may help to identify other participant genes involved of the formation of the acrosome, allowing the fine dissection of the mechanisms involved in the setup of such a specialized cellular organelle. *SPATA16* defects influence spermiogenesis, whereas meiosis is not disturbed. Thus, modulation of *SPATA16* function or that of other components in the same pathway could offer an innovative, reversible approach to male contraception that is not based on controlling the hormonal pathway of sperm production.

Table 4. Splice-Site Predictions from Three Web Sites

Web Site[a]	Odds Ratio for Sequence	
	Wild Type	Mutant
NetGene2 Server	.80	<.50
SpliceSiteFinder	.805	.681
Splice Site Prediction by Neural Network	.97	<.40

[a] See the Web Resources for URLs.

Table 5. Polymorphisms

Patient(s), Type of Globozoospermia, and Variation	Exon	Known SNP	Segregation in		Ethnicity
			Parents	Siblings	
1:					
Partial:					
c.232G→A (p.E78K)	2	Yes	…	…	European
c.397A→G (p.M133V)	2	Yes	…	…	European
2:					
Partial:					
c.232G→A (p.E78K)	2	Yes	…	…	European
c.397A→G (p.M133V)	2	Yes	…	…	European
c.1526C→T (p.A509V)	10	No	Mother	Absent in affected brother	European
c.1577T→C (p.M526T)	10	No	Mother	Absent in affected brother	European
3 and 4:					
Partial:					
c.232G→A (p.E78K)	2	Yes	…	…	European
c.397A→G (p.M133V)	2	Yes	…	…	European
5:					
Partial:					
c.232G→A (p.E78K)	2	Yes	…	Absent in affected brother	North African
c.397A→G (p.M133V)	2	Yes	…	…	…
c.440G→A (p.G147E)	2	Yes	…	…	…
7 and 8:					
Partial:					
c.232G→A (p.E78K)	2	Yes	…	Patients are brothers	European
c.397A→G (p.M133V)	2	Yes	…	…	…
c.440G→A (p.G147E)	2	Yes	…	…	…
9:					
Total:					
c.397A→G (p.M133V)	2	Yes	…	…	European
10:					
Total:					
c.232G→A (p.E78K)	2	Yes	…	…	North African
c.397A→G (p.M133V)	2	Yes	…	…	…
c.440G→A (p.G147E)	2	Yes	…	…	…

NOTE.—All nonsynonymous coding variations that were found (in 9 of 28 patients) are shown. Three known SNPs were identified, located next to two unknown but nonsegregating variations. In patients 6 and 11–28, no nonsynonymous coding variations were identified (dbSNP database).

Acknowledgments

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Web Resources

Accession numbers and URLs for data presented herein are as follows:

BLAST, http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?

dbSNP, http://www.ncbi.nlm.nih.gov/SNP/ (for exon 10 c.1526C→T [accession number *ss73688634*], exon 10 c.1577T→C [accession number *ss73688636*], and exon 4 c.848G→A [accession number *ss73688635*])

NetGene2 Server, http://www.cbs.dtu.dk/services/NetGene2/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for globozoospermia, *CSNK2A2, HRB, GOPC, SPATA16,* acrosin, and TPR)

SDSC Biology Workbench, http://workbench.sdsc.edu/ (for Biology Workbench 3.2 and ClustalW 1.81)

SpliceSiteFinder, http://www.genet.sickkids.on.ca/~ali/splicesitefinder.html

Splice Site Prediction by Neural Network, http://www.fruitfly.org/seq_tools/splice.html

UCSC Genome Browser, http://genome.cse.ucsc.edu/ (for March 2006 version)

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